DETAILED ACTION

Claim Status

1. Amendment filed on June 25, 2008 is acknowledged. Claims 1-16 are cancelled. Applicant has amended base claim 17 and added new claim 38. Currently claims 17-38 are pending and will be examined in this action.

Priority

2. Applicant's indication of where the support is found for the amendment to specification is found persuasive. Hence the amendment to the specification does not add new matter. Accordingly Applicant is entitled to the original filing date of March 7, 2003 as the priority date.

Response to Arguments

Re 103 rejection of claims 17-22, 24-31 and 33-37 over Stanton et al.; Liu et al.; Lizardi et al. and Chee et al,

3. Applicant's arguments with respect to claims 17-22, 24-31 and 33-37 have been considered but are moot in view of the new ground(s) of rejection. Applicant has amended base claim 17, to add new limitations. The added limitations change the scope of the claimed invention. The invention claimed in the instant base claim 17 is not taught by the cited art. Hence the previously cited rejections are withdrawn and new art is being introduced that teaches the amended claims.

Re 103 rejection of claims 23 and 32 over Stanton et al.; Liu et al.; Lizardi et al.; Chee et al.; in view of Bonnet et al.

4. Since rejection of base claim 17 over previously cited art is withdrawn accordingly rejection of dependent claims 23 and 32 further in view of secondary reference Bonnet et al. is no longer valid and is accordingly withdrawn.

Claim Objection

5. Claim 37 is objected to because claim 37 refers to –"said capture probe"— in claim 17. Applicant has amended claim 17 and term capture probe has been replaced by hybridization probe. Hence in the present form claim 37 lacks antecedent basis. Appropriate correction is required.

Claim Rejections - 35 USC § 103

6. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

7. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of

35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

8. Claims 17-23, 25-29, 31-32, and 34-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Spiro et al. (2000) Applied and Environmental Microbiol. vol. 66 No 10 pp 4258-4265 (NPL cited in IDS by applicant) in view of Tyagi (2000) Nature Biotechnology vol. 18 pp597-598 (NPL cited in IDS by applicant); Bonnet et al. (1999) Proc. Natl. Acad. Sci USA vol. 96, pp 6171-6176 (NPL cited in IDS by applicant); Landers (US pat. 6,844,154 B2 with priority back to April 4, 2000).

Regarding claim 17, Spiro et al. teaches a hybridization assay for at least one of a multiplicity of nucleic acid sequences in an analyte (see title and page 4258 par. 3-4) comprising the steps of:

(a) contacting said analyte with a mixture of encoded microcarriers (see page 4259 Fig 1(b) and last par. where direct hybrid capture of PCR products on bead surface--microcarriers is taught. These beads are impregnated with varying amounts of two different fluorophores—see abstract to provide encoding and page 4258 par. 3 where red and orange dyes are impregnated throughout the bead in varying amount to create different types of beads)

having immobilized on their surfaces

(i) hybridization probe for one of said multiplicity of sequences, <u>whose hybridization to said at least one sequence can be detected,</u> (see Fig. 1(b) where hybridization probe referred to as capture probe is shown attached to the surface of bead. The detection of these hybridization probes to the target is shown)

(c) determining which microcarriers have hybridization probes hybridized to said at least one nucleic acid sequence of said analyte (See page 4259 last par. section labeled direct hybrid capture of PCR product on bead surface is taught. See page 4258 par. 4. where use of flow cytometry to determining which microcarriers—encoded beads have hybridization probes (target DNA) that are labeled with green dye are hybridized to said at least one nucleic acid sequence of said analyte as they flow past the laser of flow cytometer is taught).

Regarding claim 36 Spiro et al. teach wherein said distributed array is a linear array. Spiro et al. teach sorting of beads by FACS. Here the beads are inherently sorted one after another i.e. in a linear manner hence the array of beads formed is a linear array.

Regarding claim 38 Spiro et al. teach wherein step (c) includes determining how much of said at least one nucleic acid sequence has hybridized (see title where quantitation is taught. Thus by teaching quantitation, Spiro et al. teach wherein step (c) includes determining how much of said at least one nucleic acid sequence has hybridized).

Regarding claim 17 Spiro et al. do not teach

(ii) a coding scheme comprising a plurality of signaling hairpins that are not hybridization probes for said multiplicity of sequences, including said at least one sequence: comprising quenched, fluorophore-labeled hairpin molecules each comprising an interacting affinity pair separated by a linking moiety, one member of said affinity pair having bound thereto at least one quenched fluorophore, wherein interaction of the affinity pair is disruptable to unquench said

at least one fluorophore by a physical or chemical change in a condition of its environment, wherein the disruption of the interaction of at least one affinity pair occurs at a first level of said condition and the disruption of the interaction of at least another affinity pair occurs at a second level of said condition, and wherein said disruptions are optically differentiable, and wherein the coding scheme for identifying individual microcarriers in said mixture comprises a combination of multiple spectrally differentiable fluorophores and multiple affinity pairs disruptable at detectably different levels of said condition;

(b) forming a distributed array of said microcarriers wherein location of said microcarriers in said distributed array is not used to identify said at least one nucleic acid sequence; and

(d) optically decoding the microcarriers having said at least one nucleic acid sequence hybridized to its hybridization probes to identify said at least one nucleic acid sequence by changing said condition to said detectably different levels to disrupt quenching, and detecting changes in fluorescence from the signaling hairpins.

Regarding claim 17, Tyagi teaches

(b) forming a distributed array of said microcarriers wherein location of said microcarriers in said distributed array is not used to identify said at least one nucleic acid sequence; (see page 598 2nd last par. where fluid arrays that can be analyzed by fluorescence activated cell sorters that read the optical barcode of each microbead and its cargo is taught. He also teaches an alternative method namely after hybridization the microbeads are assembled into fixed arrays that

are then interrogated by multispectral optical imaging. Thus Tyagi teaches forming a distributed array of said microcarriers wherein location of said microcarriers in said distributed array is not used to identify said at least one nucleic acid sequence because each bead is individually interrogated to determine its spectral signature to determine the nature of cargo-- identify said at least one nucleic acid sequence); and

(d) optically decoding the microcarriers having said at least one nucleic acid sequence hybridized to its hybridization probes to identify said at least one nucleic acid sequence (see page 598 last two paragraphs where optical decoding of each encoded microbead is taught).

Regarding claim 21, Tyagi teaches wherein steps (c) and (d) include decoding all microcarriers (see claim 17 above).

Regarding claims 25, 34 Tyagi teaches wherein steps (c) and (d) include flow cytometry. (see page 598 2nd last par. where Fluorescence activated cell sorter is taught. Thus teaching flow cytometry).

Regarding claims 27-28 and 31 Tyagi teaches method of claim 17 including steps a) and b) as described above. Claims 27 and 31 require that step (a) precede step (b) and claim 28 requires that step (b) precede step (a). Tyagi does teach wherein step (a) precedes step (b). (see page 598 2nd last par where hybridization to encoded microbeads preceeds formation of distributed random array). Base claim 17 has been written using open language comprising. Hence to one of ordinary skill in the art it is obvious as to the order of sequence in which these two steps are conducted. This will depend on the type of assay being

conducted. See MPEP 2144.04 IV c. 2144.04 Legal Precedent as Source of Supporting Rationale [R-1]. CHANGES IN SIZE, SHAPE, OR SEQUENCE OF ADDING INGREDIENTS. Changes in Sequence of Adding Ingredients
See *In re Burhans*, 154 F.2d 690, 69 USPQ 330 (CCPA 1946) (selection of any order of performing process steps is *prima facie* obvious in the absence of new or unexpected results).

Regarding claims 29, 35 Tyagi teaches wherein said distributed array is a planar array(see page 598 2nd last par last sentence where assembly of fixed array is taught. By teaching fixed array Tyagi teaches a planar array because microbeads are fixed on to a surface or a plane).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Tyagi in the method of Spiro et al. Spiro et al. teaches use of flow cytometery to determine identity of each microbead but Spiro does not teach forming distributed array. Tyagi teaches forming distributed array. The motivation to practice the method of Tyagi in the method of Spiro et al. to one of ordinary skill is provided by Tyagi who states "This method ---illustrates a new trend toward microarrays without positionally encoded array elements. In these arrays, probes are attached to individual microbeads distributed randomly on a flat surface or maintained in the form of a suspension. The array elements can be identified by interrogating optical barcodes that are associated with each microbead. The optical barcodes are created by imbibing the microbeads with unique combinations of differently colored fluorescent dyes at different concentrations. These “fluid” arrays can be

analyzed by fluorescence –activated cell sorters that read the optical barcode of each microbead and the identity of its cargo as it flies past the detector. Alternatively, after hybridization the microbeads are assembled into fixed arrays that are then interrogated by multispectral optical imaging. The advantage of such microbead techniques is their statistical robustness.----a high degree of redundancy can be built into the analyses, making them more reliable." (see page 598 last two par.).

Regarding claim 17, neither Spiro et al. nor Tyagi teach

(ii) a coding scheme comprising a plurality of signaling hairpins that are not hybridization probes for said multiplicity of sequences, including said at least one sequence: comprising quenched, fluorophore-labeled hairpin molecules each comprising an interacting affinity pair separated by a linking moiety, one member of said affinity pair having bound thereto at least one quenched fluorophore, wherein interaction of the affinity pair is disruptable to unquench said at least one fluorophore by a physical or chemical change in a condition of its environment, wherein the disruption of the interaction of at least one affinity pair occurs at a first level of said condition and the disruption of the interaction of at least another affinity pair occurs at a second level of said condition, and wherein said disruptions are optically differentiable, and wherein the coding scheme for identifying individual microcarriers in said mixture comprises a combination of multiple spectrally differentiable fluorophores and multiple affinity pairs disruptable at detectably different levels of said condition; and

(d) changing said condition to said detectably different levels to disrupt quenching, and detecting changes in fluorescence from the signaling hairpins.

Regarding claim 17, Bonnet et al. teaches

(ii) a plurality of signaling hairpins that are not hybridization probes for said multiplicity of sequences, including said at least one sequence (see page 6175 Table 1 where a series of molecular beacon probes—signaling hairpins that are not hybridization probes for said multiplicity of sequences, including said at least one sequence are taught):

comprising quenched, fluorophore-labeled hairpin molecules each comprising an interacting affinity pair separated by a linking moiety, one member of said affinity pair having bound thereto at least one quenched fluorophore, wherein interaction of the affinity pair is disruptable to unquench said at least one fluorophore (see page 6173 Fig. 1 where schematic of Molecular beacon is shown) by a physical or chemical change in a condition of its environment (see page 6173 par. 3 where temperature change is taught as the physical change in a condition of molecular beacon environment),

wherein the disruption of the interaction of at least one affinity pair occurs at a first level of said condition and the disruption of the interaction of at least another affinity pair occurs at a second level of said condition,(See Fig. 2 panel B phase 2 and phase 3 where see Table 1 where principle of how with increase in temperature the disruption of the interaction of at least one affinity pair occurs. See Table 1 where number of hairpins are shown that have different Tms so will be disrupted at different temperatures. Thus Bonnet et al. teaches wherein the

disruption of the interaction of at least one affinity pair occurs at a first level of said condition and the disruption of the interaction of at least another affinity pair occurs at a second level of said condition)

and wherein said disruptions are optically differentiable (see Fig. 5 top panel where melting curve of two different signaling hairpins can be optically differentiated as shown by the two curves), and

(d) changing said condition to said detectably different levels to disrupt quenching, and detecting changes in fluorescence from the signaling hairpins. (see Bonnet et al. Fig. 2 where by changing the temperature the different signaling hairpins whose Tm is equal to or lower than the actual temperature will result in disruption of the hairpin resulting in disrupted quenching, and detecting changes in fluorescence from the signaling hairpins)

Regarding claim 18, Bonnet et al. teaches wherein said interacting affinity pair comprises complementary oligonucleotide sequences hybridized to one another (see Fig. 1).

Regarding claim 19, Bonnet et al. teaches wherein said mixture of signaling hairpins includes at least three affinity pairs (see Table 1 where more than 3 affinity pairs are taught).

Regarding claim 20, Bonnet et al. teaches wherein said mixture of signaling hairpins includes from three to eight affinity pairs (see Table 1 where at least 7 affinity pairs are taught. By teaching at least 7 affinity pairs, Bonnet et al. teaches wherein said mixture of signaling hairpins includes from three to eight affinity pairs).

Regarding claim 22, Bonnet et al. teaches wherein said linking moiety comprises an oligonucleotide sequence (see Fig. 1).

Regarding claims 23, 32 Bonnet et al. teaches wherein the step of decoding includes disrupting the hybridized affinity pairs by increasing temperature (see page 6173 par. 3 and 4 where disrupting the hybridized affinity pairs by increasing temperature is taught).

Regarding claim 26, Bonnet et al. teaches wherein a quencher is attached to the complementary oligonucleotide sequence not bearing the at least one fluorophore (see Fig. 1.)

Regarding claim 37, Bonnet et al. teaches wherein said capture probe is a molecular beacon probe (see abstract).

Regarding claim 17, Bonnet et al teaches use of a quencher and fluorophore. But Bonnet et al. does not recite names of multiple spectrally differentiable fluorophores that can be used.

Regarding claim 17, Landers (see col. 17 lines 29-col. 198 lines 1-2) teaches a whole range of fluorophores. He goes on to teach the principle of how flurophore/quencher pair work (see col. 19 lines 8-24). So once the hairpin is disrupted the fluorophore would emit the light at its emission wavelength.

Thus art teaches to one of ordinary skill in the art that multiple spectrally differentiable fluorophores exist and it would have been prima facie obvious to one of ordinary skill in the art to use a suitable combination of spectrally differentiable fluorophores as taught by Landers to label different multiple affinity pairs disruptable at detectably different levels of said condition as taught by

Bonnet et al. By doing so one of ordinary skill in the art will be able to follow which affinity pair is being disrupted at what temperature by observing appropriate change in fluorescence. SEE MPEP 2144.06 Art Recognized Equivalence for the Same Purpose [R-6]. In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious. *In re Fout*, 675 F.2d 297, 213 USPQ 532 (CCPA 1982).

Thus Bonnet et al. in view of Landers teach wherein the coding scheme for identifying individual microcarriers in said mixture comprises a combination of multiple spectrally differentiable fluorophores and multiple affinity pairs disruptable at detectably different levels of said condition.

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Bonnet et al. and Landers in the method of Spiro et al. and Tyagi. The motivation to do so is provided to one of ordinary skill in the art by Bonnet et al.

Spiro et al. and Tyagi teach a hybridization assay using optically encoded microcarriers where different ratios of two fluorescent dyes that are incorporated into the microbeads determine the coding scheme. The possible permutations

that are possible using different ratios of only two dyes such that each bead can be spectrally distinguished from others is relatively limited.

Bonnet et al. show at least 7 different hairpins that differ in melting temperature from each other over 20^0C range (hairpins with temperature range of Tm taught is from 23^0C to 42^0C as shown in Table 1). By using hairpins that have different melting temperature and using spectrally distinguishable flurophores to label each of the hairpin along with the quencher, one of ordinary skill in the art can see that the number of possible uniquely encoded beads increases to 7^n where n is the number of spectrally distinguishable fluorophores based on their emission spectra. Hence by using a combination of multiple hairpins that differ in melting temperatures along with use of different fluorescence labels one of ordinary skill in the art is now able to uniquely encode many more microcarrier beads that can be optically decoded. Therefore on of ordinary skill in the art would have a reasonable expectation of success in being able to use these encoded beads in high throughput assays where massive parallel processing of multiple samples can be done using the bead format.

9. Claims 24 and 30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Spiro et al. ; Tyagi; Bonnet et al.; and Landers as applied to claims 17 and 22 above further in view of Walt et al.(2000) Science vol. 287 pp 451-452 (NPL cited in IDS by applicant).

Regarding claims 24 and 30, Spiro et al.; Tyagi; Bonnet et al.; and Landers teach method of claims 22 and 17 respectively, but regarding claims 24

and 30 they do not teach wherein forming the distributed array comprises immobilizing individual microcarriers at the ends of fibers in a fiber-optic bundle.

Regarding claims 24 and 30, Walt teaches forming the distributed array comprises immobilizing individual microcarriers at the ends of fibers in a fiber-optic bundle (see page 451 par 2).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to practice the method of Walt in the method of Spiro et al.; Tyagi; Bonnet et al.; and Landers. The motivation to do so is provided by Walt who states "A relative new comer to the array field is the self assembled bead array. This format is a departure from ----approaches and offers the molecular biologist an entirely new platform on which to study gene expression and DNA variation. The bead arrays are assembled on an optical fiber substrate". (see page 451 par. 2). They then go on to describe how the fiber optic arrays are made.

10. Claim 33 is rejected under 35 U.S.C. 103(a) as being unpatentable over Spiro et al. ; Tyagi; Bonnet et al.; and Landers as applied to claim 17 above further in view Frutos et al. (US pat. 6,579,680 B2 issued Jun 17, 2003) .

Regarding claim 33, Spiro et al.; Tyagi; Bonnet et al.; and Landers teach method of claim 17, but do not teach wherein step (d) includes disrupting said affinity pairs by adding a denaturant.

Regarding claim 33, Frutos et al. teach use of denaturants such as formamide to disrupt said affinity pairs by adding a denaturant (see col. 3 lines 48-52).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to use a denaturant (formamide) in the method Spiro et al.; Tyagi; Bonnet et al.; and Landers to disrupt said affinity pairs. Bonnet et al teaches use of temperature to disrupt said affinity pairs. Frutos et al. states" modification of certain hybridization conditions, such as temperature and use of denaturants such as formamide, that are known by those in the art to increase stringency of hybridization." (see col. 3 lines 46-52). So art teaches one of ordinary skill that changing temperature or presence of denaturant can be used to alter hybridization, hence one of ordinary skill can use the method that is desired to achieve the same end result namely disrupt said affinity pairs. SEE MPEP 2144.06 Art Recognized Equivalence for the Same Purpose [R-6]. In order to rely on equivalence as a rationale supporting an obviousness rejection, the equivalency must be recognized in the prior art, and cannot be based on applicant's disclosure or the mere fact that the components at issue are functional or mechanical equivalents. An express suggestion to substitute one equivalent component or process for another is not necessary to render such substitution obvious. *In re Fout*, 675 F.2d 297, 213 USPQ 532 (CCPA 1982).

Conclusion

11. All claims under consideration 17-38 are rejected over prior art.

12. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

13. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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